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(71) Applicant: **Ajinomoto Co., Inc.**
No. 15-1, Kyobashi 1-chome
Chuo-ku
Tokyo (JP)

(72) Inventor: **Kotani, Takuya, c/o Kawasaki Plant,**
Ajinomoto Co.
No. 1-1 Suzuki-cho,
Kawasaki-ku
Kawasaki-shi,
Kanagawa-ken (JP)
Inventor: **Lizumi, Katsuo, c/o Kawasaki Plant,**
Ajinomoto Co.
No. 1-1 Suzuki-cho,
Kawasaki-ku
Kawasaki-shi,
Kanagawa-ken (JP)
Inventor: **Takeuchi, Makoto ,c/o Kawasaki**
Plant,Ajinomoto Co.
No. 1-1 Suzuki-cho,
Kawasaki-ku
Kawasaki-shi,
Kanagawa-ken (JP)

(74) Representative: **Strehl Schübel-Hopf Groening**
& Partner
Maximilianstrasse 54
D-80538 München (DE)

(54) Method for producing L-3,4-dihydroxyphenylalanine.

(57) In a method for producing L-3,4-dihydroxyphenylalanine by subjecting microorganism cells having a β-tyrosinase activity or a product to be obtained by processing them to catalytic reaction with catechol, pyruvic acid and ammonium ion or with catechol and L-serine, the reaction is continued at a temperature lower than 25 °C under the condition where anhydrous crystals of L-3,4-dihydroxyphenylalanine exist in the reaction mixture while L-3,4-dihydroxyphenylalanine being formed are made precipitated as anhydrous crystals in the reaction mixture, and the thus-precipitated anhydrous crystals are collected.

According to the method of the present invention, crude crystals of L-3,4-dinydroxyphenylalanine having a high purity may be obtained at a high yield of recovery, by simple means.

EP 0 636 695 A1

Field of the Invention:

The present invention relates to a method for producing L-3,4-dihydroxyphenylalanine (hereinafter referred to as L-DOPA). L-DOPA has been popularly used as a medicine for curing Parkinson's disease.

Prior Art:

To produce L-DOPA, heretofore, a synthetic method starting from vanillin has been known. On the other hand, other methods for producing L-DOPA have been investigated, using an enzymatic system of microorganisms. Examples include a method for producing L-DOPA from catechol, pyruvic acid and ammonium ion, using β-tyrosinase (JP-B 48-34237 - the term "JP-B" as used herein means an "examined Japanese patent publication"), a method for producing the same from catechol and L-serine and other amino acids, also using β-tyrosinase (JP-B 47-22275), a method for producing the same from dihydroxycinnamic acid and ammonium ions, using ammonia lyase (JP-B 62-24076), a method for producing the same from L-phenylalanine or L-tyrosine, using oxygenase (JP-B 47-19033, 47-14915), a method for producing the same from 3,4-dihydroxyphenylpyruvic acid, using transaminase (JP-B 58-18475), etc.

It is known that there are two kinds of crystals of L-DOPA, including monohydrated crystals and anhydrous crystals. Regarding the crystalline morphology, monohydrated crystals of L-DOPA are needle-like crystals, while anhydrous crystals of the same are tetragonal crystals. To purify L-DOPA, there is known a method of crystallizing needle-like crystals of L-DOPA from an aqueous L-DOPA solution containing impurities at a low temperature not higher than 30 °C followed by heating them at a temperature not lower than 30 °C so as to convert them into tetragonal crystals by polymorphic transition and separating the thus-converted tetragonal crystals from the solution (JP-B 49-41188). According to the method, L-DOPA is crystallized as needle-like crystals whereby the color substances and other impurities that have been in the crude L-DOPA obtained in the production step are effectively removed. Afterwards, the needle-like crystals are separated and then converted into tetragonal crystals of pure L-DOPA by polymorphic transition.

The present inventors succeeded in the production and accumulation of an extremely high concentration of L-DOPA by improving a method of producing L-DOPA which uses β-tyrosinase (JP-A 5-123177 - the term "JP-A" as used herein means an "unexamined published Japanese patent application"). According to the improved method, L-DOPA is produced and accumulated in the reaction mixture at a concentration higher than the solubility of L-DOPA in the liquid. When, however, in this method the reaction is conducted at low temperatures, the crystals to be precipitated in the reaction system are monohydrated crystals so that the separation and the recovery of the thus-precipitated crystals are difficult. On the other hand, when the reaction is conducted at high temperatures, anhydrous crystals of L-DOPA are precipitated but many unfavorable side products are formed in the reaction system.

Problems to be Solved by the Invention:

The object of the present invention is to further improve the method of producing L-DOPA which uses β-tyrosinase derived from microorganisms so as to provide a method for producing L-DOPA more inexpensively and more efficiently than any other known conventional method of producing L-DOPA.

Means for Solving the Problem:

We, the present inventors assiduously studied the method for producing L-DOPA , which uses β-tyrosinase derived from microorganisms and, as a result, have found that L-DOPA formed by the enzymatic reaction may be precipitated as anhydrous crystals in the reaction mixture when the reaction is continued at a temperature lower than 25 °C under the condition that anhydrous crystals of L-DOPA exist in the reaction mixture and that the crystals precipitate well in the reaction mixture and therefore may easily be separated and recovered from the reaction mixture. On the basis of these findings, we have completed the present invention.

Specifically, the present invention provides a method for producing L-3,4-dihydroxyphenylalanine by catalytically reacting catechol, pyruvic acid and ammonium ion or catechol and L-serine in the presence of microorganism cells having β-tyrosinase activity or a β-tyrosinase containing product obtained by processing such cells, which is characterized in that the reaction is continued at a temperature lower than 25 °C under conditions where anhydrous crystals of L-3,4-dihydroxyphenylalanine exist in the reaction mixture while L-3,4-dihydroxyphenylalanine being formed is precipitated as anhydrous crystals from the reaction mixture, and the thus-precipitated anhydrous crystals are collected.

This means that at least during the latter part of the reaction period the reaction is carried out in the presence of anhydrous crystals of L-3,4-dihydroxyphenylalanine and at a temperature of less than 25 °C.

The microorganisms to be used in the present invention may be any of those having a β-tyrosinase (tyrosine-phenol lyase, EC 4.1.99.2) activity. As concrete examples, the following strains belonging to the genus Erwinia are preferred as they have a high activity.

Erwinia herbicola ATCC 21433

Erwinia herbicola ATCC 21434

In addition, also usable in the present invention are mutants or transformants having an improved L-DOPA productivity, which are produced by mutating the above-mentioned microorganisms or by transforming them by genetic engineering technique, etc.

For preparing cells of these microorganisms, they may be cultured in a medium containing carbon sources, nitrogen sources, inorganic salts and other nutrient substances. As the carbon source glycerol, fumaric acid, saccharides, etc. may be used. As nitrogen source ammonium sulfate, amino acids, etc. may be used. As inorganic salts potassium phosphate, magnesium sulfate, ferrous sulfate, manganese sulfate, zinc sulfate, etc. may be used. As other nutrient substances hydrolysates of soybean protein, amino acids, etc. may be used. β-tyrosinase is considered to be an adaptive enzyme, and therefore, in culturing the above-mentioned microorganisms, it is preferred to add tyrosin or substitutes for tyrosine to the medium whereby the expression of the β-tyrosinase activity in the cells is augmented to give preferable results. In addition, it is also effective to add vitamin B_6 and the like to the medium so as to elevate the β-tyrosinase activity to be expressed in the cells.

Regarding the culturing conditions, the suitable cultivation temperature is within the range of from 15 °C to 45 °C. Regarding the cultivation pH and the cultivation time, the culture is kept slightly acidic to slightly alkaline and the cultivation may be continued for 10 to 72 hours under that condition. After the microorganisms being cultured have grown to the stationary phase, the incubation may be continued for further 6 to 24 hours while the pH of the culture is maintained within a range of from 7.0 to 8.3 to obtain microorganism cells having a higher β-tyrosinase activity (JP-A-5-123177).

To carry out the catalytic reaction by contacting the microorganism cells having β-tyrosinase activity with catechol, pyruvic acid and ammonium ion or with catechol and L-serine, the thus-obtained culture may be used directly in the reaction or, alternatively, the cells may be isolated and recovered from the culture and used in the reaction. If desired, products obtained by processing the cells may also be used, including, for example, a cell homogenate, cells treated with acetone, immobilized cells, an extract from the cells, and β-tyrosinase purified from the cell extract.

According to the method of the present invention, the reaction is continued at a temperature lower than 25 °C under conditions in which anhydrous crystals of L-DOPA exist in the reaction mixture while L-DOPA being formed is precipitated as anhydrous crystals from the reaction mixture, and the thus-precipitated crystals are collected.

We, the present inventors, have found that, when microorganism cells having a β-tyrosinase activity or a product obtained by processing them are is used for producing L-DOPA, the crystals of L-DOPA which precipitate in the reaction mixture as a result of the formation and accumulation of L-DOPA therein at a concentration higher than its supersaturation solubility are monohydrated crystals at temperatures lower than about the temperature limit of 25 °C but are anhydrous crystals at temperatures higher than the same, though the limiting value depends on the composition, the pH value, etc. of the reaction mixture. The monohydrated crystals of L-DOPA have a small particle size and they are difficult to precipitate from the reaction mixture and are de-watered extremely poorly. Therefore, even if such monohydrated crystals are desired to be directly separated and recovered as crude crystals from the reaction mixture by filtration, centrifugation or the like operation, the yield of the recovery is low and the recovered crystals contain a large amount of the mother liquid adhered thereto. On the contrary, the anhydrous crystals of L-DOPA have a large particle size, and they are easy to precipitate in the reaction mixture and are de-watered well. Therefore, they may directly be separated and recovered as crude crystals from the reaction mixture by simple operation such as filtration, centrifugation or the like, and the yield of the recovery is high. In addition, the recovered crystals contain only a small amount of the mother liquid adhered thereto. Accordingly, the thus-recovered anhydrous crystals of L-DOPA may be further purified by ordinary methods such as ion-exchange resin treatment, crystallization, etc.. Given these situations, a method according to which the reaction is carried out at a temperature not lower than 25 °C, so as to make anhydrous crystals of L-DOPA precipitate in the reaction mixture, and the thus-precipitated anhydrous crystals are separated and recovered from the reaction mixture may be considered to be suitable. However, the reaction temperature not lower than 25 °C induces unfavorable side reactions by which the yield of L-DOPA is lowered. For this reason, the method is not favorable for the industrial production of L-DOPA.

According to the method of the present invention, however, the reaction is continued at a temperature lower than 25 °C under conditions where anhydrous crystals of L-DOPA exist in the reaction mixture, while L-DOPA formed by the reaction is precipitated as anhydrous crystals in the reaction mixture. Accordingly, anhydrous crystals of L-DOPA are produced at a high yield by the method of the present invention.

As one means of attaining the condition where anhydrous crystals of L-DOPA exist in the reaction mixture a method is mentioned according to which the reaction is conducted at a temperature at which L-DOPA is formed and precipitated as monohydrated crystals in the reaction mixture and anhydrous crystals of L-DOPA are added to the reaction mixture.

The temperature at which L-DOPA is formed and precipitated as monohydrated crystals in the reaction mixture is generally lower than 25 °C, though depending on the composition, the pH value, etc. of the reaction mixture. The uppermost limit of the temperature may easily be determined by experiments. Naturally, if the reaction is continued without taking any particular means for controlling the crystallization at such reaction temperature, monohydrated crystals of L-DOPA will soon precipitate in the reaction mixture. If, however, anhydrous crystals of L-DOPA are added as seed crystals to the reaction mixture prior to the precipitation of such monohydrated crystals of L-DOPA therein and the reaction is continued under this condition, L-DOPA to be formed after the addition may be precipitated as anhydrous crystals from the reaction mixture. The time when annydrous crystals of L-DOPA are added to the reaction mixture is preferably after the start of the reaction and before the precipitation of monohydrated crystals of L-DOPA to be formed by the reaction. However, even after monohydrated crystals of L-DOPA have precipitated, they may often be converted into anhydrous crystals by polymorphic transition during the reaction, without causing any problems, if a large amount of anhydrous crystals of L-DOPA exist in the reaction mixture The amount of the annydrous crystals of L-DOPA to be added may be such that the anhydrous crystals added may exist as seed crystals in the reaction mixture, and this may be determined by experiments. As one example of the addition, 0.03 g/dl of anhydrous crystals of L-DOPA are added to the reaction mixture at the time when 0.3 g/dl of L-DOPA has accumulated in the reaction mixture after the start of the reaction.

As another means of attaining the condition where anhydrous crystals of L-DOPA exist in the reaction mixture, mentioned is a method where the reaction is conducted at a temperature at which L-DOPA is formed and precipitated as anhydrous crystals in the reaction mixture, forming a reaction mixture which contains anhydrous crystals of L-DOPA therein. In this case, after the anhydrous crystals of L-DOPA have precipitated in the reaction mixture, the reaction temperature is lowered to a temperature lower than 25 °C, preferably lower than 20 °C, at which L-DOPA is generally precipitated as monohydrated crystals, and the reaction is continued at the lowered temperature. Even so, L-DOPA to be formed thereafter is precipitated as anhydrous crystals in the reaction mixture.

The temperature at which L-DOPA formed by the reaction is precipitated as anhydrous crystals also depends on the composition, the pH value, etc. of the reaction mixture, but it is generally not lower than 25 °C. The lowermost limit of the temperature may easily be determined by experiments. In this case, if the reaction is continued without taking any particular means for varying the reaction temperature, L-DOPA to be formed will precipitate as anhydrous crystals but a large amount of other side products than L-DOPA will be formed so that the yield of L-DOPA is lowered. If, however, the reaction temperature is lowered to a temperature lower than 25 °C, preferably lower than 20 °C, after anhydrous crystals of L-DOPA have precipitated in the reaction mixture, and the reaction is continued under the condition, L-DOPA to be formed thereafter is crystallized as anhydrous crystals in the reaction mixture since the previously precipitated anhydrous crystals act as seed crystals. Accordingly, the formation of side products may be prevented, and the intended L-DOPA may be obtained at a high yield.

In carrying out the method of the present invention, it is recommended to add, either continuously or intermittently, an aqueous solution containing catechol to the reaction system in such an amount that the reaction system may have a catechol concentration of 1.0% or less, in order to prevent the reaction from being inhibited by the substrate at a high concentration. Due to the addition, the speed of forming L-DOPA is elevated and the amount of L-DOPA to be accumulated in the reaction system is increased, and therefore a more favorable result may be attained (see JP-A 5-123177).

If desired, a reducing agent such as sodium sulfite or cysteine and a chelating agent such as EDTA or citric acid may be added to the reaction system. The pH of the reaction mixture is suitably within the range of from 7.7 to 8.7 and the reaction time may be determined suitably, depending on the potency and the concentration of the β-tyrosinase source used and the concentration of the substrate used.

Anhydrous crystals of L-DOPA thus obtained according to the present invention precipitate readily from the reaction mixture and may easily be de-watered. Therefore, they may easily be collected from the reaction mixture as crude crystals by filtration, centrifugation or the like operation, and the crude crystals may be further purified by ordinary methods, for example by treatment with ion-exchange resins, recrystal-

lization, etc.

Examples:

The present invention will be explained in more detail by means of the following examples.

Example 1:

One platinum loop of cells of Erwinia herbicola ATCC 21433 that had been incubated in a bouillon-agar medium at 31.5 °C for 24 hours were inoculated in 50 ml of a medium having the composition shown in Table 1 below (hereinafter referred to as a seed cultivation medium) that had been put in a 500 ml-shaking flask, and the shaking cultivation was conducted at 31 °C for 12 hours. 150 ml of the culture were transplanted in 3 liters of a medium having the composition shown in Table 2 below (hereinafter referred to as a main cultivation medium) that had been put in a 5 liter-jar fermenter, and the cultivation was conducted at 28 °C for 36 hours while the pH of the medium was kept at 7.5 by adding thereto ammonia gas and glucose. After the cultivation, the culture was divided into parts of 400 ml each. The parts were separately subjected to centrifugation to recover the cells therefrom. The thus-recovered cells were added to 300 ml of a reaction mixture for producing L-DOPA that had the composition shown in Table 3 below (hereinafter referred to as a reaction mixture) and reacted at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, separately. During the reaction, an aqueous solution containing 20% of catechol and 20% of sodium pyruvate was continuously added to the reaction system so that the catechol concentration in the reaction system was kept at 0.5% or less.

After reacted for 16 hours, the amount of L-DOPA formed was measured. Table 4 below shows the amount of L-DOPA formed and the crystal morphology of the crystals of L-DOPA precipitated.

Table 1

Components	Concentration (%)
Glycerol	1
KH_2PO_4	0.05
$MgSO_4.7H_2O$	0.05
$FeSO_4.7H_2O$	0.001
$ZnSO_4.7H_2O$	0.001
Fumaric Acid	0.2
L-tyrosine	0.2
Hydrolysate of Soybean Protein	1.5
Pyridoxine	0.01
pH 7.5 (KOH)	

Table 2

Components	Concentration (%)
Glycerol	0.5
KH_2PO_4	0.05
$MgSO_4.7H_2O$	0.05
$FeSO_4.7H_2O$	0.001
$ZnSO_4.7H_2O$	0.001
Fumaric Acid	0.7
L-tyrosine	0.2
Glycine	0.3
DL-alanine	0.3
DL-methionine	0.1
L-phenylalanine	0.2
Sodium L-glutamate	0.55
Hydrolysate of Soybean Protein	1.0
Pyridoxine	0.01
De-foaming Agent	0.002
pH 7.5 (KOH)	

Table 3

Components	Concentration (%)
Sodium Pyruvate	1.5
Catechol	1.0
Ammonium Chloride	4.0
Ammonium Nitrate	0.1
Sodium Sulfite	0.2
EDTA	0.3
pH 8.0 (aqueous ammonia)	

Table 4

Reaction Temperature (°C)	Amount of L-DOPA Accumulated (g/dl)	Morphology of Crystals	Yield (% by mol) based on Catechol
10	9.0	Monohydrated Crystals	85
15	10.0	Monohydrated Crystals	85
20	9.5	Monohydrated Crystals	75
25	7.0	Anhydrous Crystals	60
30	3.0	Anhydrous Crystals	20

Example 2:

One platinum loop of cells of Erwinia herbicola ATCC 21433 that had been incubated on a bouillon-agar medium at 31.5°C for 24 hours were inoculated in 50 ml of a seed cultivation medium having the composition mentioned above that had been put in a 500 ml-shaking flask, and the shaking cultivation was

conducted at 31 °C for 12 hours. 150 ml of the culture were transplanted in 3 liters of a main cultivation medium having the composition mentioned above that had been put in a 5 liter-jar fermenter, and the cultivation was conducted at 28 °C for 36 hours while the pH of the medium was kept at 7.5 by adding thereto ammonia gas and glucose. After the cultivation, the culture was divided into parts of 400 ml each. The parts were separately subjected to centrifugation to recover the cells therefrom. The thus-recovered cells were added to 300 ml of a reaction mixture having the composition mentioned above, and reacted at 15 °C while anhydrous crystals of L-DOPA were added thereto at the start of the reaction and 0.5, 1, 2, 4 and 6 hours after the start of the reaction, separately, each in an amount of 0.3 g/dl. During the reaction, an aqueous solution containing 20% of catechol and 20% of sodium pyruvate was continuously added to the reaction system so that the catechol concentration in the system was kept at 0.5% or less.

After reacted for 16 hours, the amount of L-DOPA formed was measured to be 10 g/dl in all of the reaction batches. Table 5 below shows the amount of L-DOPA accumulated in the reaction mixture at the indicated time when anhydrous crystals of L-DOPA had been added to the reaction mixture as well as the morphology of the final crystals of L-DOPA formed at the end of the reaction.

Table 5

Time of addition of anhydrous crystals (hr)	Amount of L-DOPA accumulated (g/dl)	Morphology of final crystals
0	0	anhydrous crystals
0.5	0.3	anhydrous crystals
1.0	1.2	anhydrous crystals
2.0	2.2	monohydrated crystals + anhydrous crystals
4.0	3.7	monohydrated crystals + anhydrous crystals
6.0	4.8	monohydrated crystals + anhydrous crystals
(not added)	-	monohydrated crystals

Example 3.

One platinum loop of cells of Erwinia herbicola ATCC 21433 that had been incubated on a bouillon-agar medium at 31.5°C for 24 hours were inoculated in 50 ml of a seed cultivation medium having the

composition mentioned above that had been put in a 500 ml-shaking flask, and the shaking cultivation was conducted at 31 °C for 12 hours. 150 ml of the culture were transplanted in 3 liters of a main cultivation medium having the composition mentioned above that had been put in a 5 liter-jar fermenter, and the cultivation was conducted at 28 °C for 36 hours while the pH of the medium was kept at 7.5 by adding thereto ammonia gas and glucose. After the cultivation, the culture was divided into two parts of 400 ml each. The both parts were separately subjected to centrifugation to recover the cells therefrom. The thus-recovered cells were added to 300 ml of a reaction mixture having the composition mentioned above and reacted at 25 °C. During the reaction, an aqueous solution containing 20% of catechol and 20% of sodium pyruvate was continuously added to the reaction system so that the catechol concentration in the system was kept at 0.5% or less. With the proceeding of the reaction, L-DOPA began to precipitate as annydrous crystals. In one reaction batch of the two, the reaction temperature was lowered to 15 °C after annydrous crystals of L-DOPA began to precipitate and the reaction was continued at the lowered temperature. In the other reaction batch, the reaction was continued still at 25 °C even after anhydrous crystals of L-DOPA began to precipitate.

After 16 hours, anhydrous crystals of L-DOPA were finally obtained in the both reaction batches. In the former batch where the reaction temperature was lowered, the yield of the product was 9.5 g/dl. In the latter batch where the reaction temperature was not lowered, however, the yield of the product was 7.0 g/dl. The yield of the product to catechol was 80% in the former batch, while that to catechol was 60% in the latter batch.

Example 4:

One platinum loop of cells of Erwinia herbicola ATCC 21433 that had been incubated on a bouillon-agar medium at 31.5 °C for 24 hours were inoculated in 50 ml of a seed cultivation medium having the composition mentioned above that had been put in a 500 ml-shaking flask, and the shaking cultivation was conducted at 31 °C for 12 hours. 25 ml of the culture were transplanted in 25 liters of a seed cultivation medium having the composition mentioned above that had been put in a 50 liter-jar fermenter, cultured therein at 31 °C for 16 hours, then transplanted in 500 liters of a main cultivation medium having the composition mentioned above that had been put in a 800 liter-jar fermenter, and further cultured therein at 28 °C for 36 hours while the pH of the medium was kept at 7.5 by adding thereto ammonia gas and glucose. After the cultivation, the cells were recovered from the culture by centrifugation, and they were divided into two parts. Each part was added to 200 liters of a reaction mixture having the composition mentioned above, and reacted at 15 °C. During the reaction, an aqueous solution containing 20% of catechol and 20% of sodium pyruvate was continuously added to the reaction system so that the catechol concentration in the system was kept at 0.5% or less. To one reaction batch of the two, added were anhydrous crystals of L-DOPA in an amount of 0.5 g/dl when the concentration of L-DOPA formed and accumulated in the reaction system reached 0.3 g/dl after the start of the reaction, and the reaction was continued further. To the other reaction batch, anhydrous crystals of L-DOPA were not added and the reaction was continued further. After 20 hours, the amount of L-DOPA formed was 10.0 g/dl in both of the two reaction batches. Anhydrous crystals of L-DOPA precipitated in the former reaction batch, while monohydrated crystals of L-DOPA formed in the latter reaction batch. The crystals formed were separated from 200 liters of each of the two reaction batches, using Sharpless Superdecanter P660 Mode (made by Tomoe Industrial Co.), and slurries of crude crystals were recovered. The recovery of L-DOPA from the former reaction batch where anhydrous crystals of L-DOPA had precipitated was 96%, and the water content in the slurry of crude crystals recovered was 26%. However, the recovery of L-DOPA from the latter reaction batch where monohydrated crystals of L-DOPA had formed was only 28%, and the slurry of crude crystals recovered contained 63% of water. One kg of the slurry of crude crystals of L-DOPA that had been recovered as anhydrous crystals was dissolved in an acid to remove the cells therefrom by an ordinary method, and the crystals were adsorbed to active charcoal in a column and then eluted with a dilute aqueous ammonia containing 0.2% of sodium sulfite. After neutralized and concentrated, the eluate was recrystallized three times each with water. Thus, 350 g of pure crystals of L-DOPA were obtained.

Claims

1. A method for producing L-3,4-dihydroxyphenylalanine by catalytically reacting catechol, pyruvic acid and ammonium ion or catechol and L-serine in the presence of microorganism cells having β-tyrosinase activity or a product obtained by processing these cells,

characterized in that at least during the latter part of the reaction period the reaction is performed

at a temperature lower than 25 °C under the condition that anhydrous crystals of L-3,4-dihydroxyphenylalanine exist in the reaction mixture, while L-3,4-dihydroxyphenylalanine is formed and precipitated in the form of anhydrous crystals, and the thus precipitated anhydrous crystals are collected.

2. The method according to claim 1, characterized in that preformed anhydrous crystals of L-3,4-dihydroxyphenylalanine are added to the reaction mixture while the reaction is conducted at a temperature at which L-3,4-dihydroxyphenylalanine normally precipitates in the form of monohydrated crystals.

3. The method according to claim 1, characterized in that anhydrous crystals of L-3,4-dihydroxyphenylalanine are precipitated in the reaction mixture by conducting the reaction at a temperature at which L-3,4-dihydroxyphenylalanine precipitates in the form of anhydrous crystals, and thereafter the reaction is continued at a temperature at which L-3,4-dihydroxyphenylalanine normally precipitates in the form of monohydrated crystals.

4. The method according to any of the claims 1 to 3, wherein the product obtained by processing microorganism cells having β-tyrosinase activity is a cell homogenate, cells treated with acetone, immobilized cells, a cell extract or purified β-tyrosinase.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 94111423.3

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,P, A	PATENT ABSTRACTS OF JAPAN, unexaminded applications, C field, vo. 17, no. 482, September 2, 1993 THE PATENT OFFICE JAPANESE GOVERNMENT p 106 C 1105 * No. 05 123 177 * --	1	C 12 P 13/22
A	DE - A - 1 960 524 (AJINOMOTO) * Claims * & JP-B-47 22 275 --	1	
A	DE - A - 2 152 548 (AJINOMOTO) * Pages 2-4 * & JP-B-48 34 548 --	1	
A	US - A - 3 813 317 (BENOITON et al.) * Abstract; claims 1,3,9 * ----	1	TECHNICAL FIELDS SEARCHED (Int. Cl.5) C 12 P

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	11-10-1994	WOLF

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
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E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

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